

Tuomeya Harvey, a genus of freshwater Rhodophyta newly recorded from China

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Abstract *Tuomeya* Harvey (*Tuomeya americana* (Kützinger) Papenfuss) is reported as new record to China. The specimens were collected from Xuzhou in Jiangsu, East China. This genus is characterized by a cartilaginous thallus and solid cortex, which is composed of numerous compact cortical cells. Its carpogonial branches are twisted. In addition to describing the morphological and reproductive structures of *T. americana* in detail, we also use *rbcL* gene sequences aligned with the previously published sequences of *T. americana* from North America. *Tuomeya* includes only one species, which was previously known from North America and South Africa.

Key words *Tuomeya*, Batrachospermaceae, Batrachospermales, freshwater Rhodophyta, *rbcL* gene, new record, China.

While collecting plants of freshwater Rhodophyta from Xuzhou (34°12'02.82" N, 117°15'46.11" E) in Jiangsu, a small cartilaginous plant belonging to the Batrachospermales was obtained that was different from other reported genera and species in China. The specimen was identified as *Tuomeya americana* (Kützinger) Papenfuss and represents a new record for China. We described its morphological characters. In addition, its chloroplast ribulose-1, 5-bisphosphate carboxylase/oxygenase (*rbcL*) gene was sequenced and aligned with the previously published sequences of *T. americana* from North America (Vis et al., 1998; Kapraun et al., 2007).

1 Material and methods

The materials were collected on rocks and stones in flowing water from Bajian Spring, Xuzhou, Jiangsu, on 24 March, 2006. Specimens used for the morphological study were preserved in freshwater containing 4%–10% formalin, and those used for molecular study were frozen at –20 °C. The vegetative and reproductive structures were examined with a light microscope (Olympus BX51) and with a dissecting microscope (Olympus SZ30). Photographs were taken with a camera (Olympus C5060) attached to the microscope. Specimens used in this study were deposited in the Herbarium of Shanxi University (SXU).

For DNA analysis, the frozen samples were ground in liquid nitrogen, and DNA was extracted following the protocol described by Saunders (1993), except that samples were purified using the Wizard DNA Clean-up System (Promega, Madison, Wisconsin, USA.) according to the manufacturer's protocol. Polymerase chain reaction (PCR) amplification was performed in 580BR thermocycler (2686, BIO-RAD, USA). The following cycle was used for amplification: initial denaturation at 95 °C for 5 min, 35 cycles at 94 °C for 1 min, 60 °C for 1 min and 72 °C for 2 min, and a final extension at 72 °C for 2 min. The primer pair F160 (5'CCT CAA CCA GGA GTA GAT CC3') and *rbcL* reverse (5'ACA TTT GCT GTT GGA GTC TC3') was used to amplify a 1282 bp fragment of *rbcL* gene. PCR products were

prepared for sequencing with the Gel Extraction Mini Kit (Huashun, Watson Biotechnologies, Inc., Shanghai, China) according to the manufacturer's protocol. The PCR products were sequenced using the BigDye[®] Terminator v3.1 Cycle Sequencing Kit [Applied Biosystems (ABI), Foster City, CA, USA], ABI Prism[™] 3730XL DNA Analyzer and ABI Prism[™] 377XL DNA Sequencer. Sequencing reactions were prepared and sequenced with the amplification primers in two directions.

The sequences were compared with other *T. americana* from USA, which had been deposited in GenBank (Vis et al., 1998; Kapraun et al., 2007). The sequences were aligned using CLUSTAL X software, and final adjustments were made by eye. The MEGA (version 3.0) program was used to calculate sequence statistics (Kumar et al., 2004).

2 Results

2.1 Morphological descriptions

Tuomeya Harvey in Smithsonian Contributions to Knowledge 10 (2): 64. 1858.——*Batrachospermum* section *Tuomeya* (Harvey) Necchi & Entwisle in Phycologia 29: 478. 1990.

托氏藻属

Thalli are irregularly branched, cartilaginous, and solid. Plants are composed of longitudinal axial cell filament with numerous whorls of lateral branches and cortical filaments. Spermatangia are ovoid, terminating on lateral branch. Carpogonium-bearing branches are short, arising from periaxial cells or cells of fascicles. Carpogonia are asymmetrical with elongate-conical or club-shaped trichogynes. Carposporophytes are definite in shape. Gonimoblast filaments are the radially branched type, arising from the dense mass of fusion cells (gonimoblast placenta), including the original involucre cells.

Type: *Tuomeya americana* (Kützinger) Papenfuss.

Tuomeya americana (Kützinger) Papenfuss in Taxon 7: 104. 1958; Kaczmarczyk et al. in Journ. Phycology 28: 850, figs. 2–10, 1992; Kumano in Freshwater Red Algae of the World 238. 2002. ——*Baileya americana* Kützinger in Tab. Phycol. VII: 35, pl. 87, III, f, f', g. 1857. ——*Tuomeya fluviatilis* Harvey in Smithsonian Contributions to Knowledge 10 (2): 64. 1858; Setchell in Proc. Amer. Acad. Arts & Sci. 25: 53. 1890; Skuja in Acta Horti Bot. Univ. Latv. 14: 3. 1944; Starmach in Flora Slodkowodna Polski. 214. 1977.

美洲托氏藻 Figs. 1–3

Plants are densely branched, monoecious, caespitose, cartilaginous and cylindrical gametophytes. It ranges in color from blue-green to olive, and plants are about 2.5–5 cm high (Fig. 1: A). Three to five layers of cortical filaments, dense laterals arising from pericentral cells, cover the uniaxial filament (Fig. 1: C; 3: F). Cortical cells are long cylindrical, 40–50 μm long, 8.5–9.5 μm in diameter (Fig. 1: D; 3: A). Axial cells are evident in mature branches by their constrictions. Whorls are not well developed, remote from each other, about 35–40 μm in diameter (Fig. 1: B). Internodes are about 85–105 μm long. Primary fascicles are 1–3 dichotomously branched, 5–7 cells long, and cells are cylindrical-ellipsoid, 18–20 μm long, 9–11 μm in diameter, with rare terminal hairs. Secondary fascicles are also 1–3 dichotomously branched, and cells similar to the primary fascicles.

Carpogonia are asymmetrical with an irregularly broadened trichogyne attached obliquely or perpendicularly to a stalk and born on a curved carpogonial branch derived from basal cells. Carpogonial branches are twisted, 7–10 cells long, and each cell is 2.5–4 μm long, 5 μm in diameter (Fig. 2: B; 3: E). Carpogonia (including the trichogyne) are 45–55 μm long, 10–12 μm in diameter (Fig. 2: D; 3: C). The trichogyne is long, ellipsoid. Carposporophytes are discretely shaped, globose, 200–250 μm in diameter (Fig. 2: C), and 1(–2) per whorl.

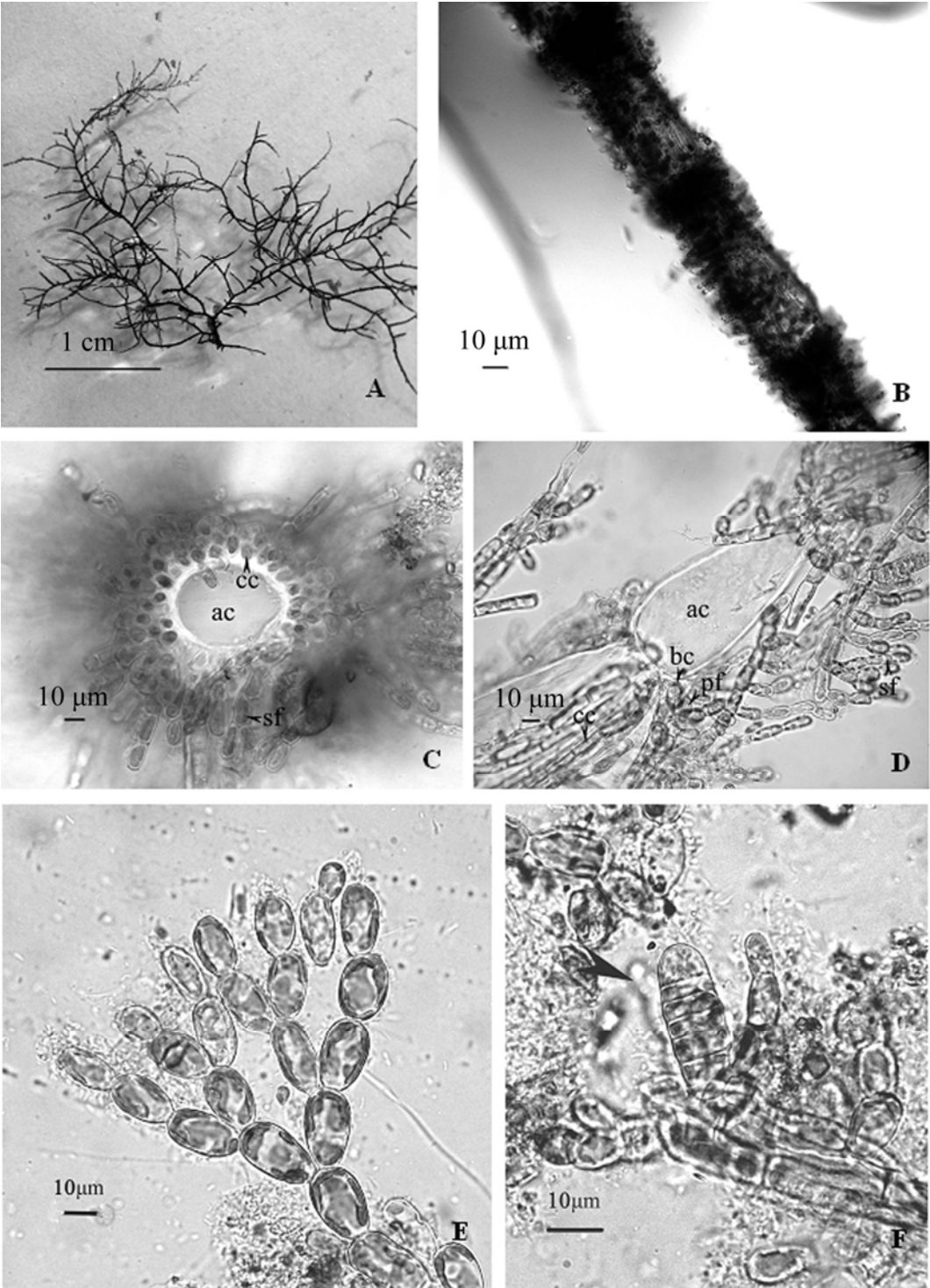


Fig. 1. *Tuomeya americana* (Kützinger) Papenfuss. **A**, Habit of frond. **B**, Part of a frond, showing shape of whorls. **C**, The cross section through a node showing an axial cell and numerous layers of cortical cells. **D**, Numerous cortical filaments. **E**, Carposporangia. **F**, Apical portion of branch with apical cell (arrow).
Ac, axial cell; bc, basal cell; cc, cortical cell; pf, primary fascicle; sf, secondary fascicle.

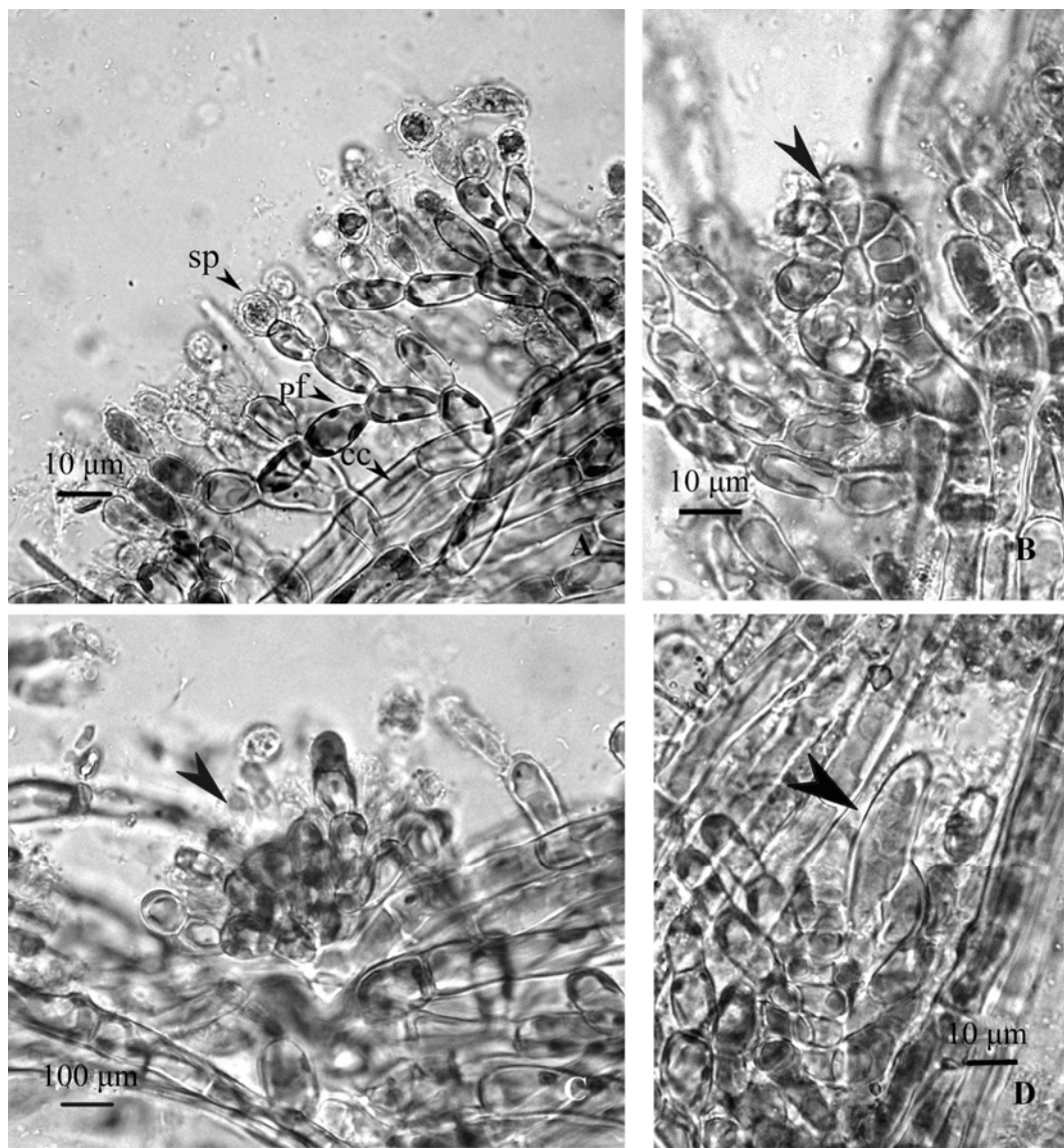


Fig. 2. *Tuomeya americana* (Kützinger) Papenfuss. **A**, Fascicle with terminated spermatangia. **B**, Juvenile coiled carpogonial branch (arrow). **C**, Carposporophyte, showing carposporangia (arrow). **D**, Mature carpogonium (arrow). Cc, cortical cell; pf, primary fascicle; sp, spermatangium.

Carposporangia are ovoid, 15–21 μm in length, 12–15 μm in diameter (Fig. 1: E; 3: D). Spermatangia are globose, single or double, terminating lateral branches, 6.5–7.5 μm in diameter (Fig. 2: A; 3: B).

China. Jiangsu (江苏): Xuzhou (徐州) (34°12'02.82" N, 117°15'46.11" E), collected in Bajian Spring, on rocks and stones in flowing water, 2006-03-24, *J. Feng et al.* (冯佳等) JS2006001 (SXU).

Distribution: Eastern North America from Newfoundland in the north to Alabama and Louisiana in the south (Kaczmarczyk et al., 1992; Vis et al., 1998; Kapraun et al., 2007) and South Africa (Borge, 1928).

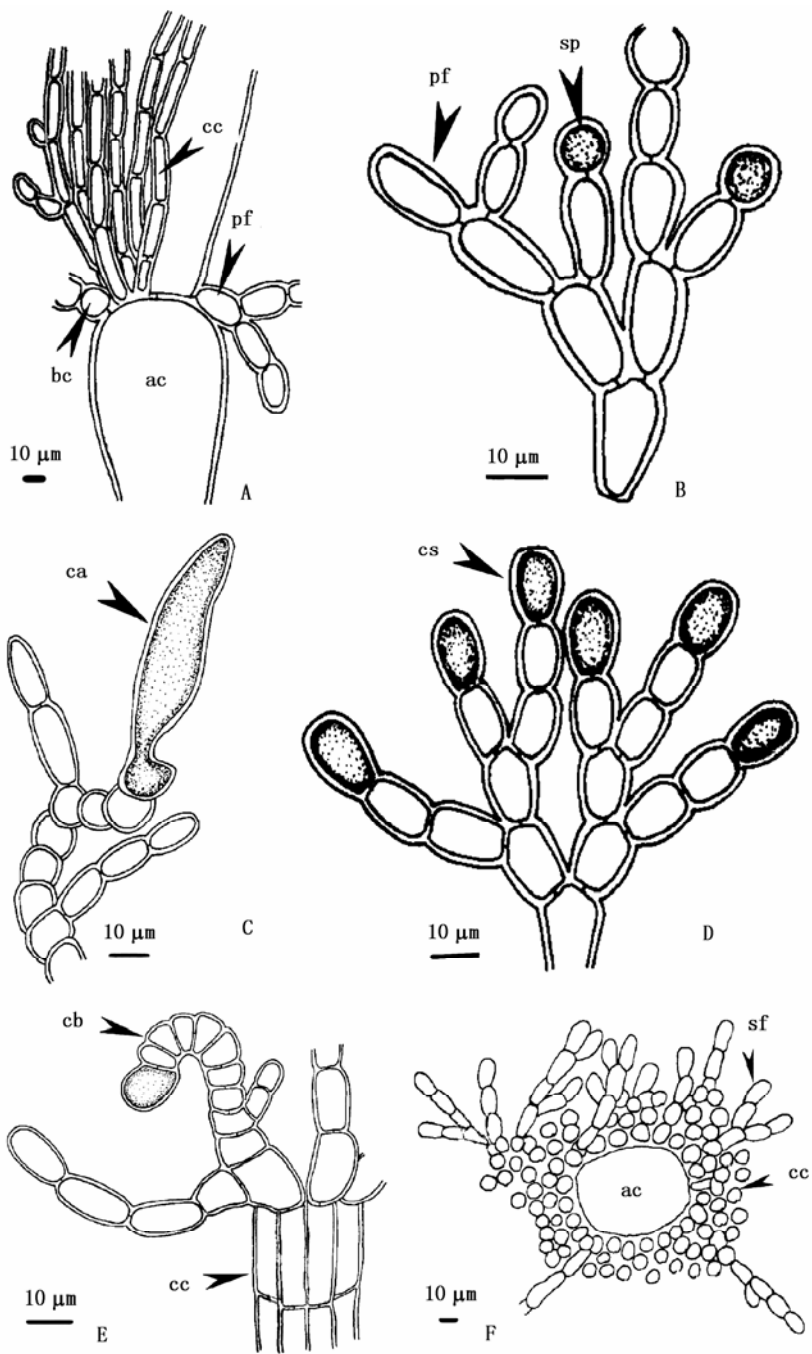


Fig. 3. *Tuomeya americana* (Kützinger) Papenfuss. A, axial cells and cortical cells; B, fascicle with terminated spermatangia; C, carposporangia; D, carposporangia; E, juvenile coiled carposporangium; F, the cross section through a node showing numerous layers of cortical cells. Drawn by S. L. Xie from J. Feng et al. JS2006001.

Ac, axial cell; bc, basal cell; ca, carposporangium; cb, carposporangium; cc, cortical cell; cs, carposporangia; pf, primary fascicle; sf, second fascicle; sp, spermatangium.

Fig. 4. Sequence alignment of *rbcL* region of the chloroplast DNA for *Tuomeya americana* populations sampled. A dash indicates a deletion relative to the Xuzhou collection, and N indicates a missing base.

Table 1 List of samples information and accession numbers of *rbcL* sequence of *Tuomeya americana* in GenBank

Sample	Collection information	Accession number
1	USA: Beaver River, South Kingston, Rhode Island	AF029352
2	USA: Lower Barton Creek, North Carolina	AF029159
3	China: Xuzhou, Jiangsu	
4	USA: Lower Barton Creek, North Carolina	DQ523253

3 Discussion

The genus *Tuomeya* belongs to Batrachospermaceae, Batrachospermales, Rhodophyta. It was originally distinguished from the other members of Batrachospermales by its having a densely branched, cartilaginous, and pseudoparenchymatous thallus with compacted lateral whorls radiating from a uniseriate axis (Harvey, 1858). Subsequently, Setchell (1890) noted that the plants appeared to be intermediate between *Batrachospermum* and *Lemanea*. Necchi and Entwisle (1990) proposed that *Tuomeya* should be reduced to a section of the genus *Batrachospermum*, but in 1992, Kaczmarczyk, Sheath and Cole confirmed the previous classification scheme because *Tuomeya* differs from *Batrachospermum* in its pseudoparenchymatous growth and its carpogonia with obliquely to perpendicularly attached trichogynes. Moreover, *Tuomeya* is unique in having gametophyte development from a basal mass of undifferentiated cells. Kaczmarczyk et al. (1992) also used multivariate morphometrics and concluded that the genus *Tuomeya* should be maintained as distinct in contrast to the proposal of Necchi and Entwisle. We considered that the classification scheme supported by Kaczmarczyk et al. is much more reasonable.

When the genus *Tuomeya* was described by Harvey in 1858, *T. fluviatilis* (specimen collected by Tuomey in Alabama, USA) was designated as the type species. However, this taxon had been earlier validated as *Baileya americana* by Kützing (1857). Papenfuss (1958) renamed the species as *T. Americana* based on an older name of Kützing.

The amplified *rbcL* region of chloroplast DNA of *T. americana* obtained from Xuzhou is comprised of 1282 bp and a BLAST search gives close resemblance of this sample with the others collected from the North America (GenBank accession number AF029159) having 85.57% similarity. Between the population from Xuzhou and North Carolina (DQ523253) there was 14.51% sequence variation, and in pairwise comparisons of sequence data of 721 bp, there were 95 bp changes (13.18% sequence divergence) between collections from Xuzhou and the sample (AF029352). However, the sequence divergence values are 0.55%–10.37% among previously published sequence from North America. Therefore, the divergence among them may result from the biogeographical isolation.

Tuomeya americana was previously known only from North America and South Africa. It is reported from China (and Asia) for the first time.

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中国淡水红藻一个新记录属——托氏藻属

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摘要 报道了中国淡水红藻一个新记录属——托氏藻属*Tuomeya* Harvey。标本采于江苏徐州。该属以植物体软骨质，多层细胞紧密排列组成坚固的皮层，果胞枝弯曲等特征，区别于串珠藻科Batrachospermaceae的其他属。除对此种藻类的形态作详细描述外，还通过*rbcL*序列比较分析其与北美地区样本的地理差异。该属先前在加拿大、美国和南非有过报道。

关键词 托氏藻属；串珠藻科；串珠藻目；淡水红藻；*rbcL*序列；新记录；中国